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Properties of Thalidomide and its Analogues: Implications for Anticancer Therapy

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ABSTRACT

Thalidomide and its immunomodulatory (IMiDs) analogs (lenalidomide, Revlimid, CC-5013; CC-4047, ACTIMID) are a novel class of compounds with numerous effects on the body's immune system, some of which are thought to mediate the anticancer and anti-inflammatory results observed in humans. Thalidomide is currently being used experimentally to treat various cancers and inflammatory diseases. It is approved for the treatment of dermal reaction from leprosy and is currently in phase III trials for multiple myeloma. Thalidomide and IMiDs inhibit the cytokines tumor necrosis factor-α (TNF-α), interleukins (IL) 1β, 6, 12, and granulocyte macrophage-colony stimulating factor (GM-CSF). They also costimulate primary human T lymphocytes inducing their proliferation, cytokine production, and cytotoxic activity thereby increasing the T cells' anticancer activity. They induce an IL-2-mediated primary T cell proliferation with a concomitant increase in IFN-γ production and decrease the density of TNF-α−induced cell surface adhesion molecules ICAM-1, VCAM-1, and E-selectin on human umbilical vein endothelial cells. Thalidomide stimulates the Th-1 response increasing IFN-γ levels while CC-4047 increased IL-2 as well. Some of the above immunomodulatory activities along with anti-angiogenic, anti-proliferative, and pro-apoptotic properties are thought to mediate the IMiDs' antitumor responses observed in relapsed and refractory multiple myeloma and some solid tumor cancers. This has led to their use in various oncology clinical trials. The second generation IMiD, lenalidomide, has shown potential in treating the bone marrow disorders myelodysplastic syndrome and multiple myeloma. It is currently in phase II and III trials for these diseases respectively with numerous phase II trials in other hematologic and solid tumors.

KEYWORDS: Thalidomide, lenalidomide, IMiDs, immunomodulatory, cytokine, multiple myeloma, anti-cancer.

HISTORY

In August 1998, thalidomide (Figure 1) was approved for sale in the United States for the chronic treatment of erythema nodosum leprosum (ENL), a painful inflammatory dermatologic reaction of lepromatous leprosy.[1] This marked the approval of the world's most controversial drug after it was withdrawn from Europe more than 40 years ago. For an excellent account of the tortuous history of thalidomide, I refer the reader to the recent book by Stephens and Brynner.[2] Thalidomide was first synthesized in Germany in 1954 from the glutamic acid derivative α-phthaloylisoglutamine and soon thereafter animal studies showed it to be extremely nontoxic. It was erroneously concluded that the purported structural resemblance to the then widely used barbiturates could indicate its potential as a "safe" sedative. A single questionable study in mice was performed to show its sedative-hypnotic effects.[2] Based on this study, human trials were initiated in Germany under the lax pharmaceutical regulatory environment without comprehensive animal toxicology studies. These studies should have included reproductive toxicology in a non-rodent species such as rabbits or monkeys. Thalidomide was found to be an effective sedative and sleep-inducing agent in humans with less potential for overdose compared with the barbiturates. It was approved in Germany in 1957 and subsequently in other countries including the United Kingdom, Canada, and Australia under brand names such as Contergan, Distaval, Talimol, and Kevadon. Thalidomide was also found to be an effective anti-emetic in pregnancy and its use in this group of patients subsequently increased. The error in this presumption of good efficacy with limited toxicity became apparent when reports of deformed babies started appearing from late 1956. By the time it was withdrawn in 1961, ~5000 to 12 000 deformed babies (and an unknown number of aborted fetuses) from 46 countries were already born.[3] Thalidomide was never approved in the United States because of the diligence of the Food and Drug Administration (FDA) reviewer Frances Kelsey who requested more information from the petitioning company on the reported peripheral neuritis.[4] The company was not forthcoming and the application was withdrawn. This would have been the end of the drug were it not for subsequent reports of its effectiveness in treating various inflammatory and dermatological conditions such as ENL. Thalidomide was found to be so effective in treating the skin lesions associated with ENL that it is now the World Health Organization's recommended drug for this form of leprosy. With therapeutic use of thalidomide increasing in the United States, its potential for fetal toxicity is a major concern. The lowest doses and shortest treatment period where characteristic birth defects in human fetuses have been documented were 25 mg/day (0.5 mg/kg based on a 50-kg human) for 2 to 3 days and 50 mg/day (1 mg/kg/day) for only 1 day.[5] To reduce this potential, thalidomide's marketing and use is restricted through the mandatory System for Thalidomide Education and Prescribing Safety (STEPS) program.[6] This unique system of monitoring oversees the prescribing, dispensing, and dosing of thalidomide. All patients, pharmacists, and prescribing physicians must be registered in Celgene's database. First-time patients are required to view a video from a thalidomide victim warning them of the teratogenicity of thalidomide.

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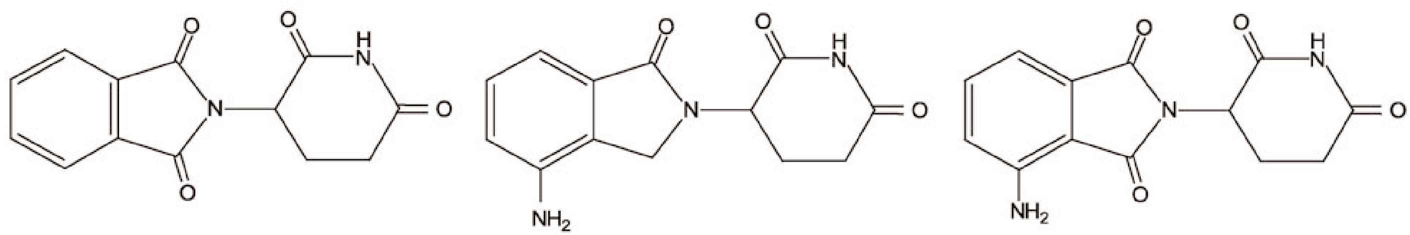

Figure 1. Structures of thalidomide, lenalidomide and CC-4047.

All sexually active patients are required to use 2 forms of contraception. Prescriptions are only for a 28-day supply with renewals requiring a visit to the physician and completion of a questionnaire on sexual activity.[6] The STEPS program therefore makes thalidomide the most restrictively prescribed drug ever approved.

This review highlights and presents some of the activities observed with thalidomide and its IMiD (immunomodulatory drugs) analogs, especially their effects on tumor necrosis factor-α (TNF-α), interleukins (IL), interferon gamma (IFN-γ), and various lymphocytes. Our review extends these observations by presenting more recent thalidomide and IMiD activity in inducing natural killer (NK) cells. We also present the compounds' surprising anti-cancer activity in myelodysplastic syndrome, multiple myeloma, and other cancers and discuss the role of immuno- and non-immunomodulatory mechanisms.

Current Human Trials and Experimental Uses of Thalidomide

Apart from its approved use in ENL, thalidomide is also prescribed experimentally, mostly co-administered with standard therapies, in more than 150 clinical trials in the United States for various oncologic, dermatologic, and inflammatory conditions.[7-11] While therapeutic doses are 100 to 400 mg/day orally (2 to 8 mg/kg/day) for dermatologic and inflammatory diseases, various oncologic indications have been using 200 to 400 mg/day as maintenance doses (4 to 8 mg/kg/day). Potential efficacy has been observed in human trials for relapsed/refractory multiple myeloma,[9,12] mantle cell lymphoma,[10] glioma,[13] metastatic melanoma,[14] and pancreatic cancer.[15] Confirmatory studies are in progress in some of these cancers. Thalidomide is currently being considered for approval in newly diagnosed multiple myeloma by the FDA. Treatment in heavily pretreated relapsed multiple myeloma patients showed a total response rate of 32%.[9] In addition, combination treatment with thalidomide and dexamethasone in newly diagnosed multiple myeloma induced response rates of 80% vs 53% for dexamethasone alone.[12] Adverse events associated with thalidomide use, some of which may be dose-related, include somnolence, constipation, rash, peripheral neuropathy, and deep vein thrombosis.[16]

IMiDs are Potent Analogues of Thalidomide Currently in Human Trials

Various analogs of thalidomide have been synthesized and screened for anti-cancer and anti-inflammatory activities. These orally administered analogs are called IMiDs for their numerous effects on the body's immune system. Lenalidomide (Revlimid; CC-5013) and CC-4047 (Actimid) are second-generation thalidomide analogs currently in clinical trials. Both analogs have potent immunomodulatory activities.[17] In in vitro studies, thalidomide has been shown to inhibit the cytokine, TNF-α, in human monocytes.[18]

Using TNF-α inhibition as a basis for comparison, lenalidomide and CC-4047 are 2000 and 20 000 times more potent than thalidomide respectively.[19] TNF-α inhibition is one of many mechanisms of action in thalidomide and the IMiD's efficacy in multiple myeloma (see below).

Multiple myeloma is an incurable B or plasma cell malignancy of the bone marrow accounting for 1% to 2% of all cancers and 10% of new hematological malignancies in the United States and is diagnosed in ~15,000 patients annually in the United States. It is characterized by the secretion of monoclonal proteins or immunoglobulins (M protein or paraprotein). Most patients die within 5 years of diagnosis because of limited treatment options and the relapsing and refractory nature of the disease.[20] Lenalidomide at 5 to 50 mg/day has been shown to overcome conventional drug resistance in relapsed multiple myeloma patients with no thalidomide-like side effects (sedation, constipation, peripheral neuropathy).[21] Myelosuppression characterized by reductions of white blood and platelet counts, however, was observed at 50 mg/day requiring dose reduction or "drug holiday." Seventeen of 24 patients (71%) responded positively to treatment with at least 25% reduction in paraprotein levels. Lenalidomide is currently in phase III trials for relapsed multiple myeloma. Significant activity has also been seen in another bone marrow disease called preleukemia or myelodysplastic syndrome (MDS) affecting 13 000 new patients each year. In MDS, bone marrow function is abnormal with not enough normal blood cells being made. The most common sign is anemia, which may require blood transfusion. MDS may eventually change into acute myeloid leukemia.[22] Preliminary studies showed that lenalidomide at 10 or 25 mg/day or 10 mg/day for 21 days with a 7-day treatment-free period produced significant responses in patients with low and intermediate risk MDS. Erythroid response was observed in 21 of 33 patients (64%) with major response (transfusion-independence) seen in 19 patients.[23] Significant cytogenetic response was seen in a subset of MDS patients exhibiting 5q- chromosomal deletion. Lenalidomide is currently in phase II trials for MDS. CC-4047 has recently showed promising activity in patients with metastatic hormone-refractory prostate cancer.[24] Six of 13 patients had significant decrease in prostate-specific antigen at a dose of 1 mg/day. Toxicity included constipation, nausea, and fatigue. Expanded trials at 2 mg/day are ongoing.

Figure 2. Efficacy of thalidomide in an ENL patient.

Thalidomide and the IMiDs Have Potent Immunodulatory Properties

Inhibition of TNF-α, IL-1β, IL-6, IL-12, GM-CSF, and Stimulation of IL-10 in Peripheral Blood Mononuclear Cells

Cytokines are soluble glycoproteins released by cells of the immune system that act non-enzymatically through specific receptors to regulate immune responses. TNF-α is a proinflammatory cytokine produced by monocytes, macrophages, lymphocytes, and NK cells.[17] It plays an important role in host immune and inflammatory response to viral, parasitic, fungal, and bacterial infections. TNF-α has been implicated in the pathogenesis of infections and autoimmune diseases. Elevated levels of TNF-α have been associated with various inflammatory and immune disorders such as rheumatoid arthritis, Crohn's disease, tuberculosis, cancer cachexia, and ENL. Thalidomide's ameliorative effects on ENL have been particularly striking (Figure 2). Thalidomide and its analogs are potent inhibitors of TNF-α production by lipopolysaccharide-stimulated human monocytes.[25] This inhibition is due to the increased degradation of TNF-α mRNA by thalidomide.[26] Levels of other cytokines, IL-1β, IL-6, and granulocyte macrophage-colony stimulating factor (GM-CSF), are also inhibited by thalidomide, whereas IL-10 is stimulated.[27] Lenalidomide and CC-4047 also had similar effects on these cytokines, although with varying degrees of potency compared with thalidomide. The effects of these findings on various diseases are still being investigated.

Costimulation of Primary Human T Cell Number and Activity

Thalidomide has been shown to costimulate primary human T lymphocytes, inducing their proliferation, cytokine production, and cytotoxic activity.[28] Costimulation involves the delivery of a second signal to naïve T cells to produce an antigen-specific response. Thalidomide's immunologic adjuvant action therefore stimulates the otherwise ineffectual immune response, for example to tumor antigens enhancing the anticancer response. This thalidomide action is, however, dependent on the type of immune cell that is activated and the type of stimulus the cell receives. In in vitro studies, thalidomide induced an IL-2–mediated primary T cell proliferation through the T cell receptor (TCR) complex with a concomitant increase in IFN-γ production.[27] The proliferation is greater for the cytotoxic rather than helper T cell subset. This finding is supported by observations in thalidomide-treated HIV-seropositive patients where it increased the population of cytotoxic T cell and plasma levels of IL-2 receptor, a marker of T cell activation. The stimulatory property may partially explain thalidomide's anti-inflammatory effects in inflammatory bowel disease in which the activity of cytotoxic T cells is diminished. Thalidomide's effects depend on the disease and immunologic status. Its costimulatory activity explains the unexpected increase in TNF-α production in certain diseases.[29] IMiDs are more potent than thalidomide in costimulating T cells that have been partially activated by the TCR. For example, the costimulatory action of CC-4047 is thought to produce the prolonged antitumor response seen in mice implanted with colorectal cancer cells.[29] Protection is thought to be mediated by T helper cell-1 (Th-1) cellular immunity (see below).

Modification of Surface Cell Adhesion Molecules

In response to an inflammatory stimulus, leukocytes are recruited into the injured tissue by capture, rolling, tight binding, transmigration across the endothelium, and chemotaxis. Thalidomide has been shown to decrease the density of TNF-α–induced cell surface adhesion molecules ICAM-1, VCAM-1, and E-selectin on human umbilical vein endothelial cells.[30] L-selectin was also decreased by thalidomide in vitro. Blocking of this adhesion cascade by thalidomide is thought to mediate the antivasculitis effect seen with ENL. This decrease in cell adhesion molecule expression is also thought to occur with multiple myeloma. Lenalidomide decreases binding of multiple myeloma cells to the endogenous bone marrow stromal cells thereby decreasing the production of vascular endothelial growth factor (VEGF) and IL-6 (see below). Evidence suggests modulation of adhesion molecules between these 2 cell types.[21]

Stimulation of Th-1 Immunity and Associated Increased IL-2 and IFN-γ Secretion

Thalidomide has been shown to cause a stimulation of the Th-1 response in healthy humans after oral dosing. This was manifested by an increase in IFN-γ without changes in IL-2 and IL-4 levels.[31] In murine models of colorectal cancer and melanoma, CC-4047 increased the Th-1 cytokines IFN-γ and IL-2.[32] In scleroderma patients, incubation of their peripheral blood mononuclear cells with thalidomide produced a dose-dependent increase in IFN-γ and IL-2.[33] Subsequent studies of the IMiDs showed that the increase in T cell cytokine production is through potentiation of the transcription factor activator protein-1.[34] The results in healthy humans and scleroderma patients strongly suggest enhancement of Th-1–type immune activity by thalidomide.

Induction of NK Cell Activity and Number

Thalidomide and IMIDs produced significantly increased lysis of human multiple myeloma cell lines and patient multiple myeloma cells after incubation with IL-2 primed peripheral blood mononuclear cells.[35] This activity was found to be mediated by NK cells. Patients with relapsed multiple myeloma had increased absolute number of NK cells with only responding patients showing an increase in the percent of such cells. This increase in NK cells was accompanied by a decrease in the plasma disease biomarker paraprotein and an increase in IL-2 and IFN-γ secretion.[35] These findings correlate with the known Th-1 stimulatory effects of thalidomide and IMiDs as discussed earlier. Elevated levels of these Th-1 circulating cytokines stimulated the activity and number of NK cells leading to the observed lysis of multiple myeloma cells.

Nonimmunomodulatory Properties

Anti-angiogenic Activity

Angiogenesis is the development of new blood vessels. In cancer this can nurture the growth and metastasis of tumors and tumor cells respectively. Thalidomide and IMiDs has been shown to have antiangiogenic properties that are independent of their immunomodulatory effects.[36,37] This activity is thought to play a role in the apparent efficacy seen with various cancers. In the rat aorta assay, the IMiDs were found to be 2 to 3 times more potent in their antiangiogenic activity compared with thalidomide. Lenalidomide but not thalidomide and CC-4047 significantly inhibited the migration of endothelial cells. CC-4047 on the other hand inhibited VEGF but not basic fibroblast growth factor (bFGF). The IMiDs' anti-TNF-α activity had no effect on anti-angiogenic activity.[37] In multiple myeloma, the close proximity interaction between the indigenous bone marrow stromal cells and patient multiple myeloma cells significantly increased levels of the pro-angiogenic factors VEGF and IL-6 (a multiple myeloma growth and survival factor).[21,29,35,38,39] Thalidomide and CC-4047 significantly decreased expression of these factors thereby reducing the production and growth of new blood vessels feeding the multiple myeloma cells.[38] These findings underscore the importance of stromal and multiple myeloma cell interaction in the bone marrow microenvironment for the maintenance and progression of the disease and provide another target for thalidomide and its analogs.

Anti-proliferative and Pro-apoptotic Activity in Tumor Cells

Thalidomide and the IMiDs produce less than 20% and 50% inhibition of DNA synthesis respectively in human multiple myeloma cell lines and cells from patients.[39] The IMiDs also inhibited the proliferation of doxorubicin- and melphalan-resistant multiple myeloma cells by 20% to 50%.[21,29] These results correlate well with the anti-tumor activity seen in patients with the drug-resistant forms of the disease. The anti-proliferative mechanism of action is thought to be by inhibition of IL-6 production. IMiDs have pro-apoptotic activity in human multiple myeloma cells. They arrest cell growth at the G1 phase and trigger activation of caspase-8, enhance multiple myeloma cell sensitivity to Fas-induced apoptosis, and downregulate nuclear factor (NF)-κB activity as well as the expression of apoptosis inhibitory protein.[39,40]

Activity of Thalidomide and IMiDs in Multiple Myeloma

Thalidomide was serendipitously found to have anti-myeloma activity when it was thought its anti-angiogenic activity could slow the disease by inhibiting the formation of new blood vessels in this highly vascularized cancer. There is now ample evidence to show that the anticancer activity of thalidomide and its analogs in multiple myeloma is through different mechanisms and sites in the bone marrow.[21,39] Figure 3 shows the bone marrow microenvironment in multiple myeloma, which contains aberrations in various cellular processes, immunology, and cell interactions. Thalidomide and the IMiDs' immunomodulatory activities consist of inhibiting the expression of IL-6 and TNF-α by bone marrow stromal cells that in turn inhibit the growth of multiple myeloma cells. The compounds also enhance T cell stimulation and proliferation with the activated cells, then releasing IL-2 and IFN-γ. These cytokines activate NK cells causing lysis of the multiple myeloma cells.[29,39] The combination of immunomodulatory and non-immunomodulatory anticancer activities in the bone marrow is thought to produce the significant anti-tumor responses observed in some multiple myeloma patients. This combination activity has significant implications for other blood and solid tumor cancers and is currently being investigated in numerous clinical trials.

Activity of Thalidomide and IMiDs in Solid Tumors

Phase II trials of thalidomide have shown potential activity against some solid tumors.[8,14,15] While the mechanism is unclear, it is thought to involve both immunomodulatory and non-immunomodulatory activities. These tumors produce immunologic suppressive factors that prevent priming and activation of CD4+ and CD8+ T cells of the lymph nodes.[27] Other immune cells such as NK and macrophages are also inhibited and are therefore unable to respond to and destroy

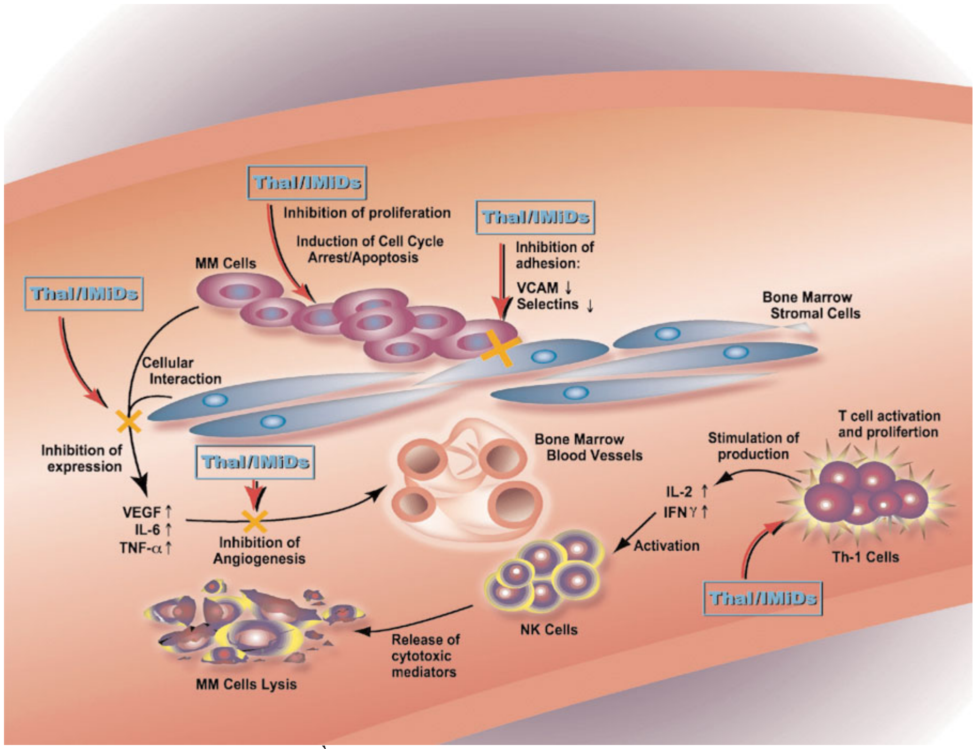

Figure 3. Sites of activity of thalidomide and IMiDÒs in the bone marrow of multiple myeloma patients.

tumor cells.[27] Thalidomide and the IMiDs' costimulatory action on primary human T cells enhance antitumor activity mediated by the Th-1 cytokines IL-2 and IFN-γ. The costimulation is thought to overcome the T cell unresponsiveness and prevent the release of suppressive factors, thereby enabling tumor-specific cells to kill tumor cells.[27,28] Thalidomide and the IMiDs are also thought to costimulate macrophages and NK cells leading to antitumor activity as discussed earlier.[35] Anti-angiogenic and pro-apoptotic activities of thalidomide and the IMiDs are also thought to play a role in the apparent efficacy seen in various highly vascularized solid tumors through inhibition of VEGF and induction of growth arrest respectively.[29,39]

Future of Thalidomide and Its Analogues

The rehabilitation of thalidomide has increased its experimental use in numerous oncologic and inflammatory conditions. It has yet to be approved for multiple myeloma. Recent reports of potential efficacy in other solid tumors have increased its experimental use and initiation of further clinical trials. The future of this class of compounds is in the more potent IMiD analogs, particularly lenalidomide. Their significantly increased immunomodulatory and anti-angiogenic potency and apparent lack of thalidomide's side effects have made them potentially important therapeutics in cancer. In addition, lenalidomide is not teratogenic in rabbit, a sensitive species for thalidomide-induced birth defects, making lenalidomide a more desirable therapeutic drug candidate than its parent. The IMiDs therefore represent second generation small molecule compounds with novel mechanisms of anticancer activity. Their potential as anticancer therapeutics is enormous should their activities be confirmed in ongoing phase II and III trials.

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